FIG. 1

MLV-based transduction using Cre/loxP system as previously described

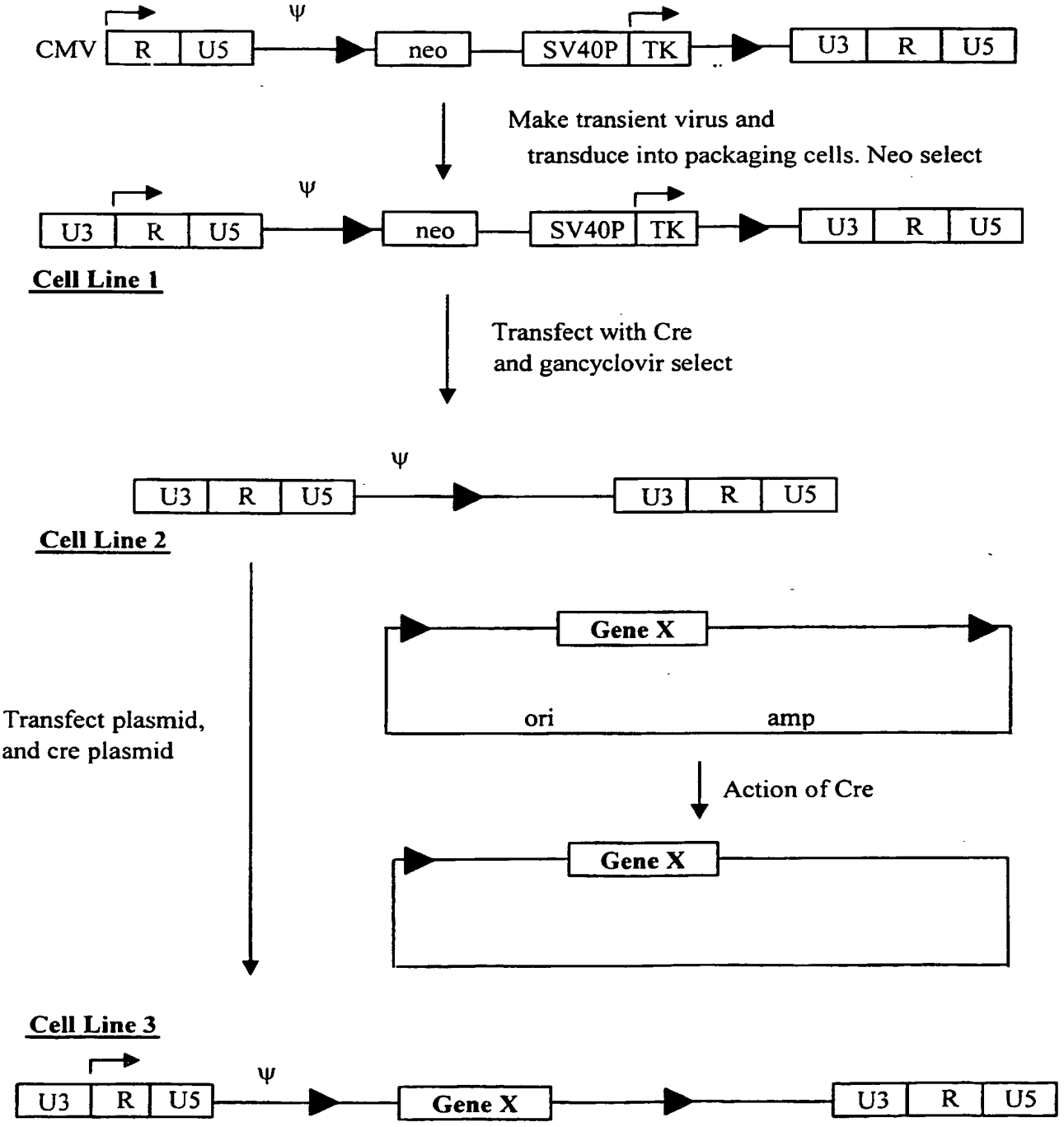


FIG. 2

EIAV-based transduction Cre/loxP system

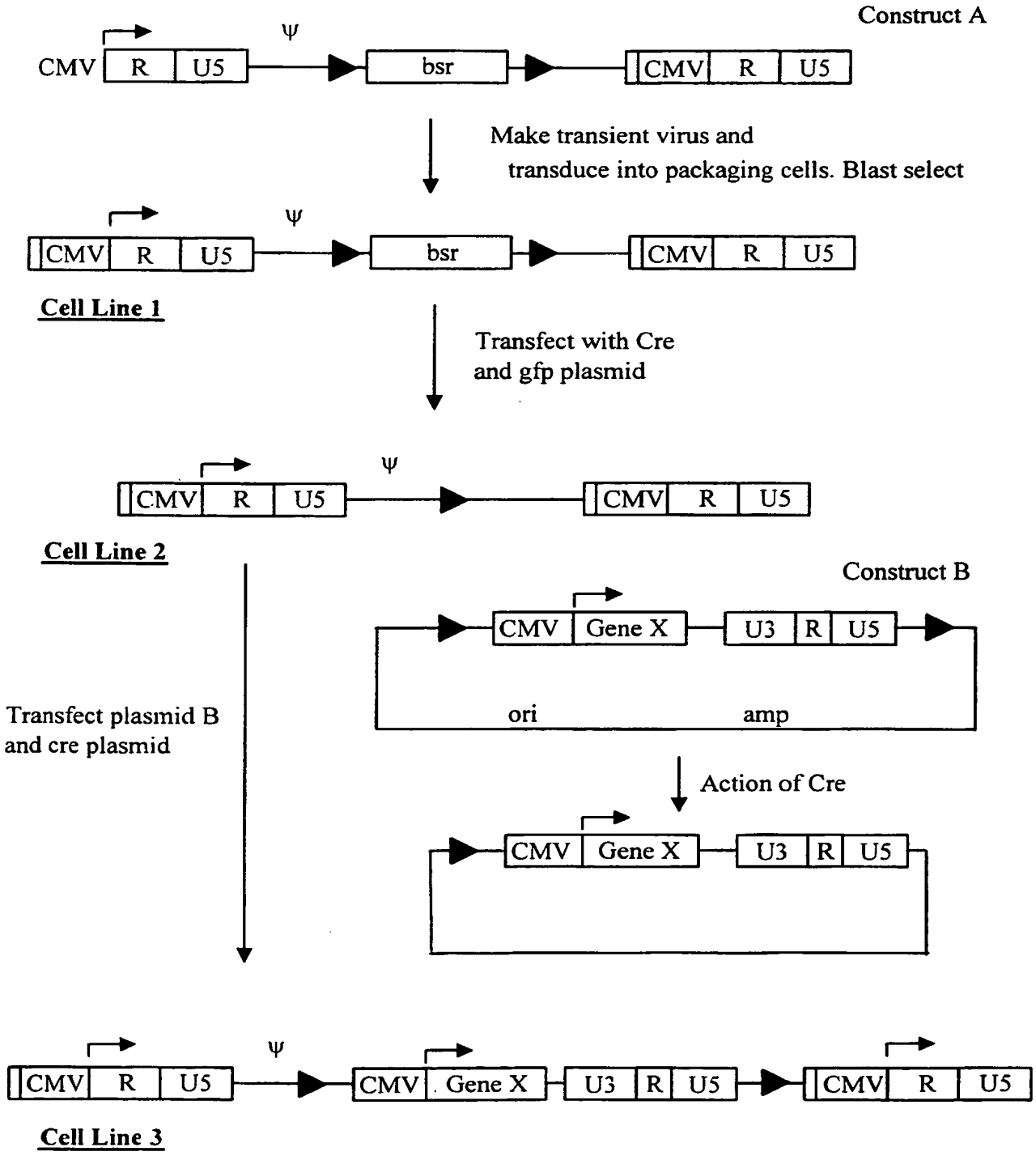


FIG. 3

MLV SIN vector approach, with EIAV components in blue

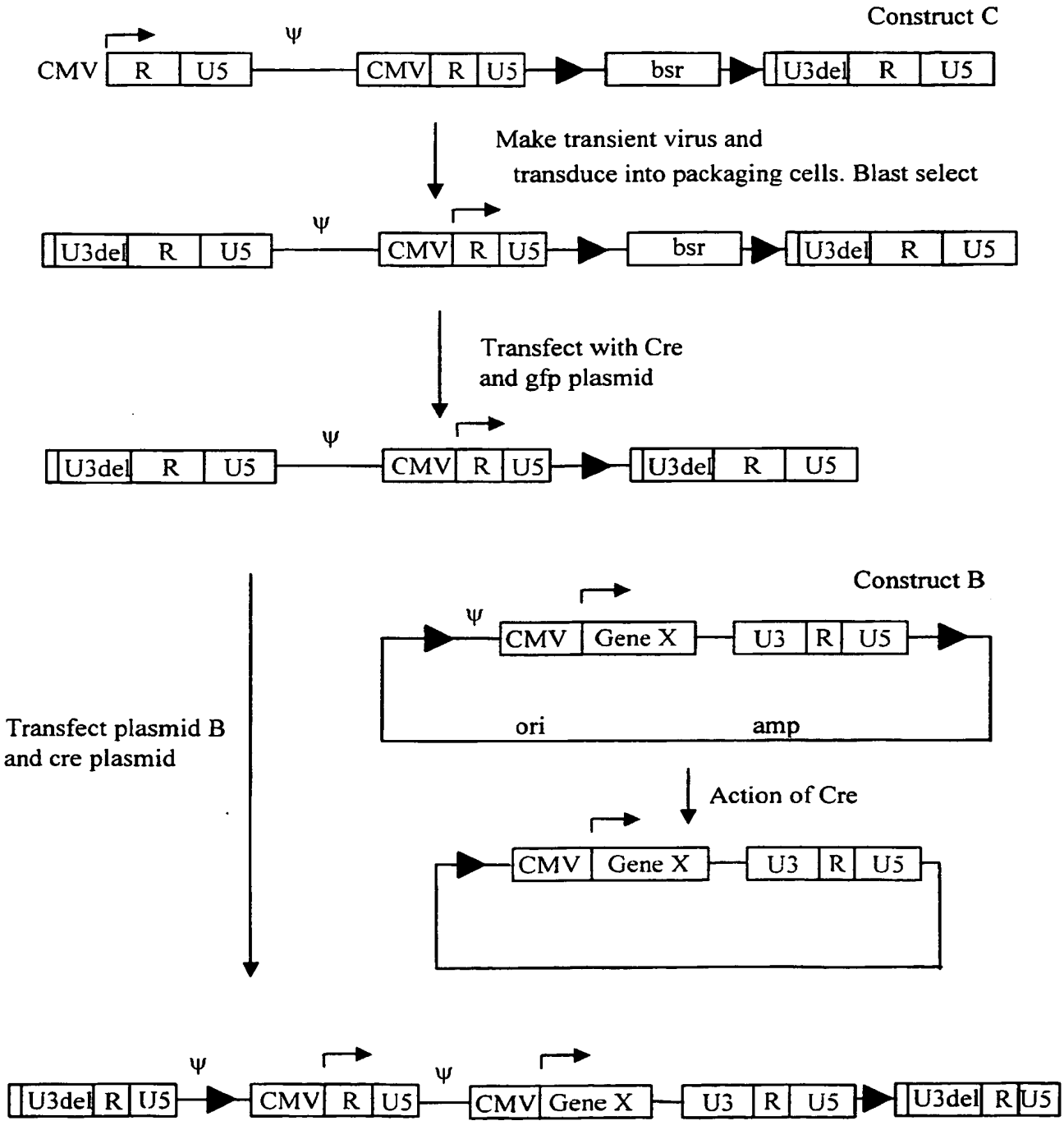


FIG. 4

MLV-based transduction with HRE 3' LTR using Cre/loxP system

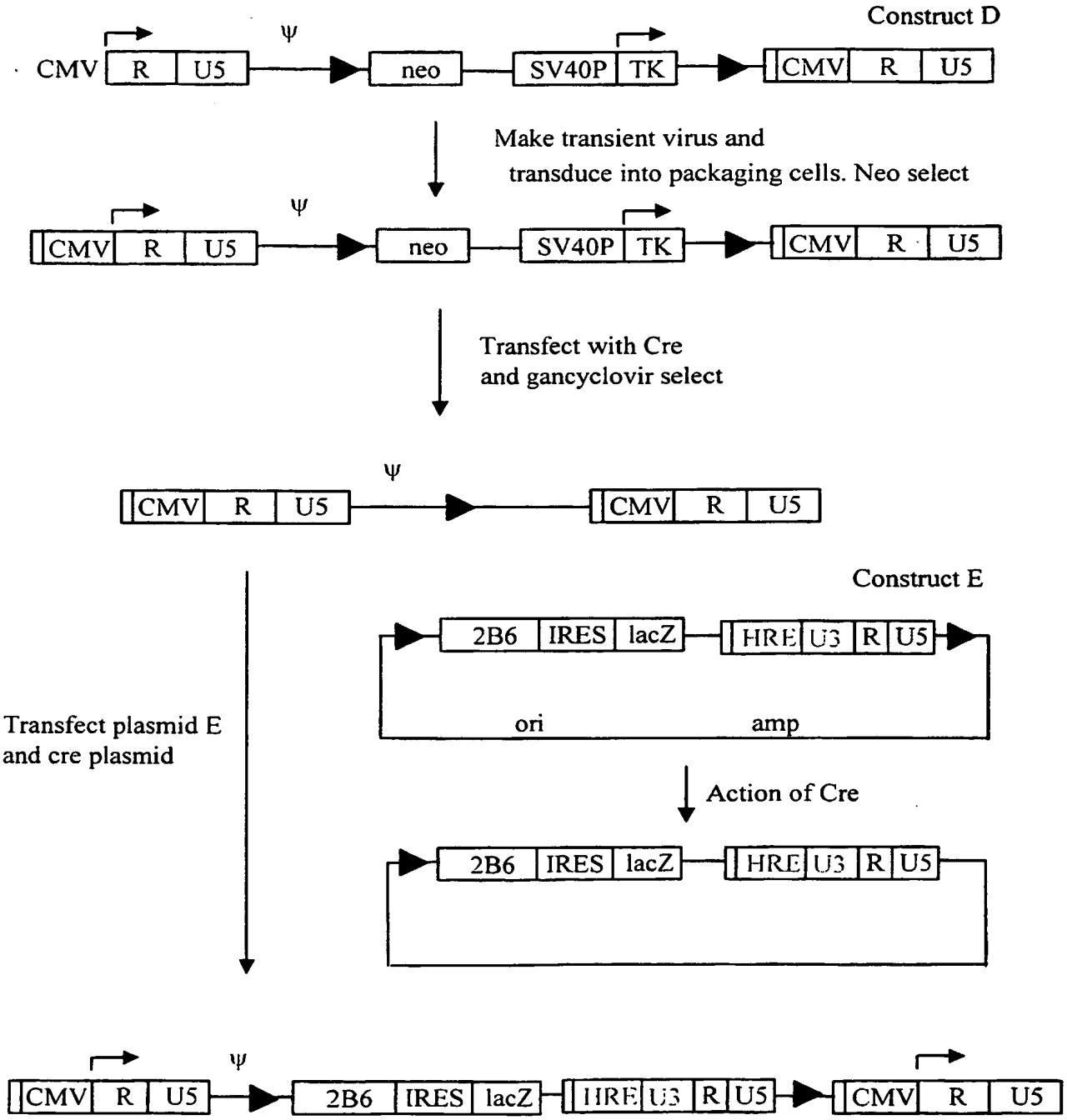


FIG. 5

MLV-based transduction for SIN vector production using Cre/loxP system

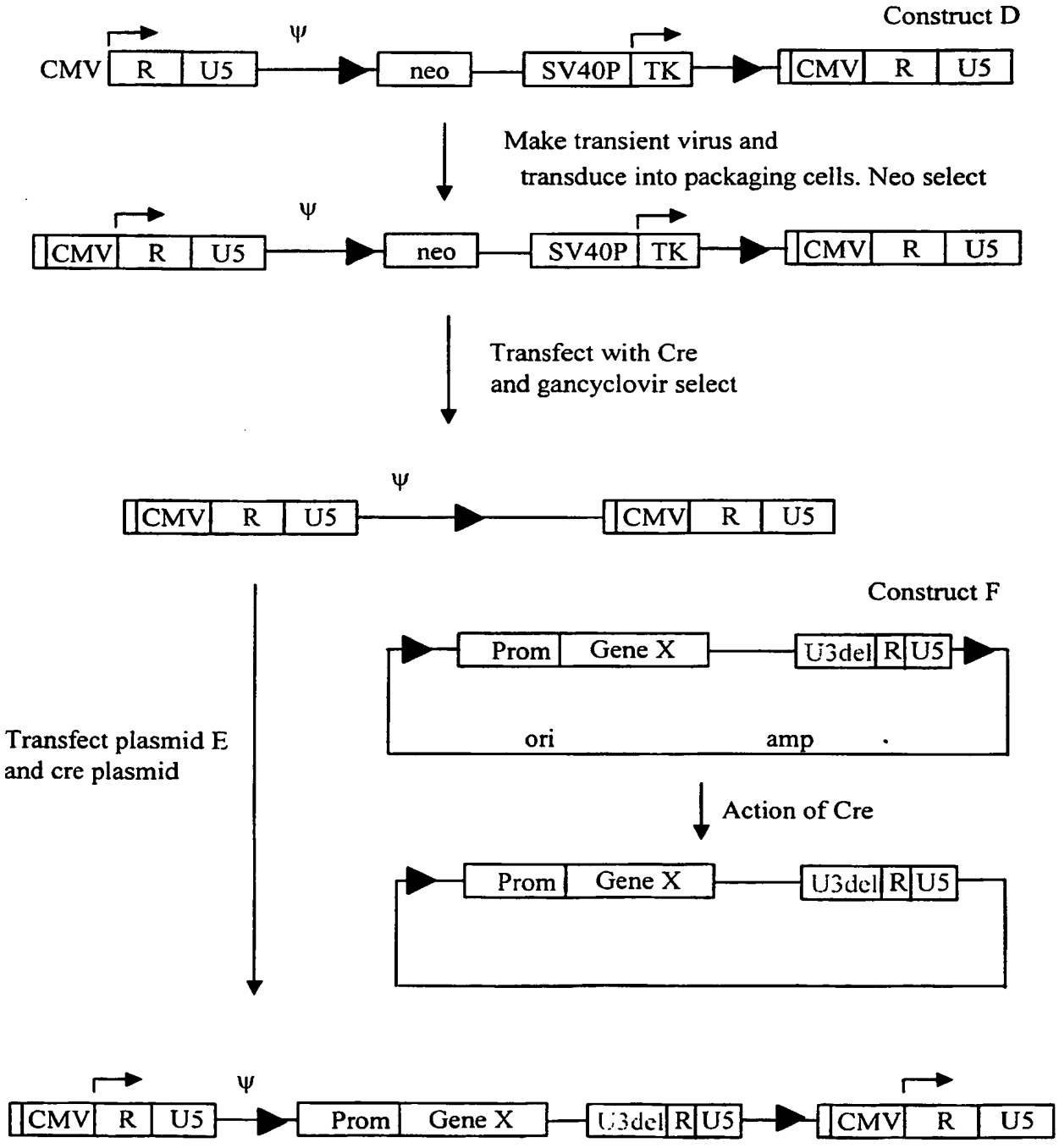


FIG. 6

MLV SIN-vector based transduction system. This general approach can be used with EIAV, HIV or MLV genomes

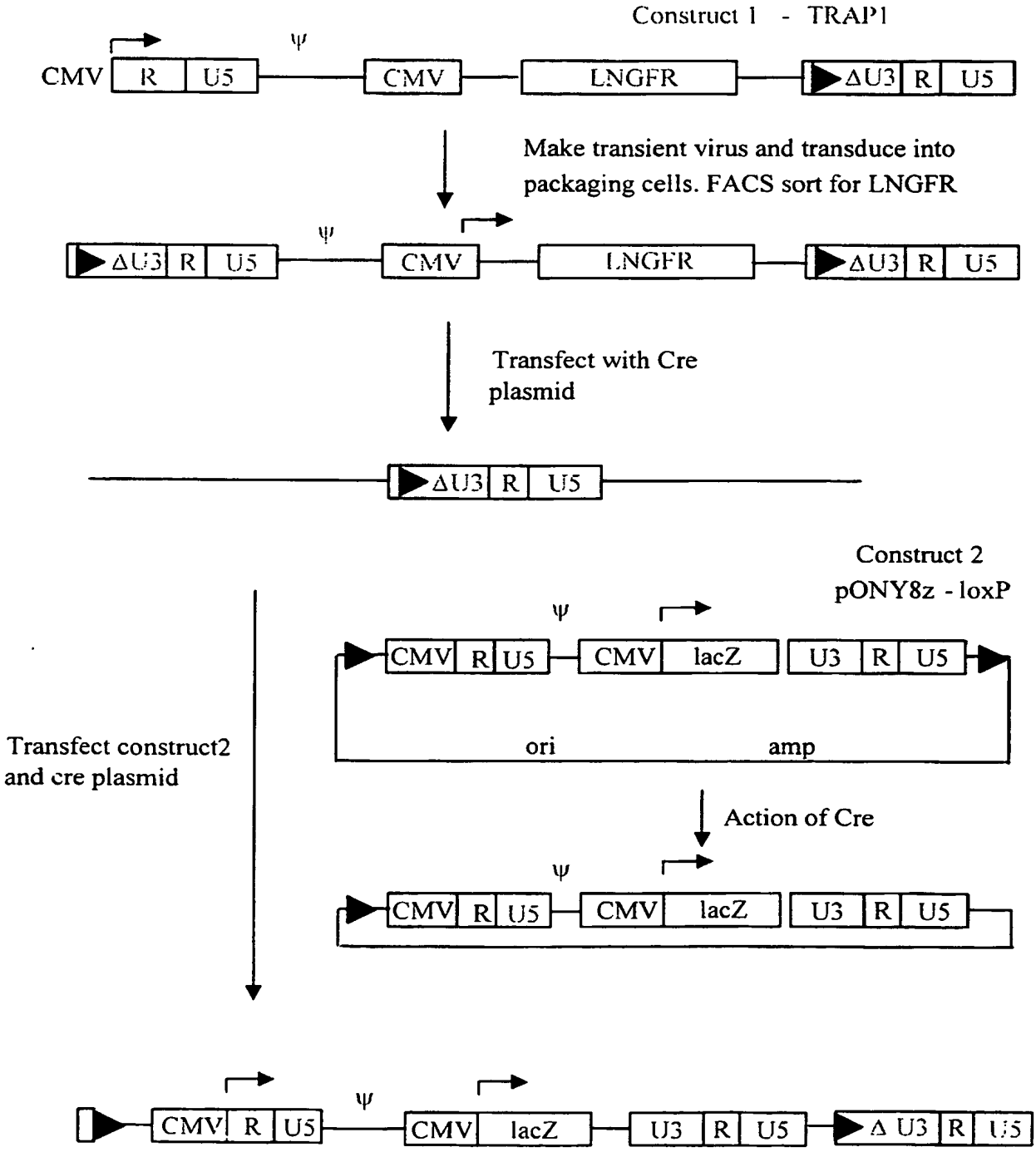


FIG. 7

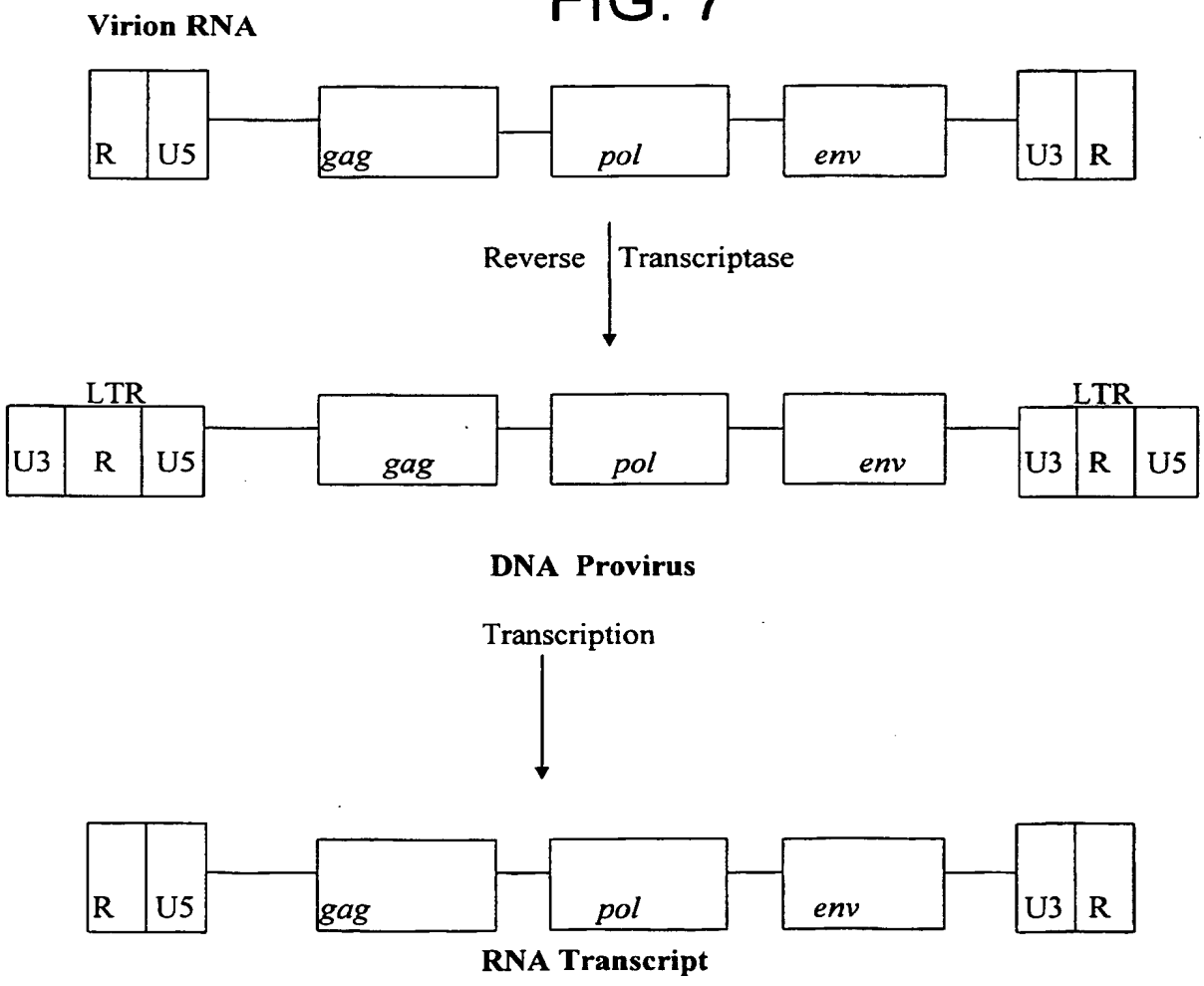

Virion RNA
R
U5
gag
pol
env
U3
R
Reverse Transcriptase
LTR
U3
R
U5
gag
pol
env
LTR
U3
R
U5
DNA Provirus
Transcription
R
U5
gag
pol
env
U3
R
RNA Transcript

FIG. 8

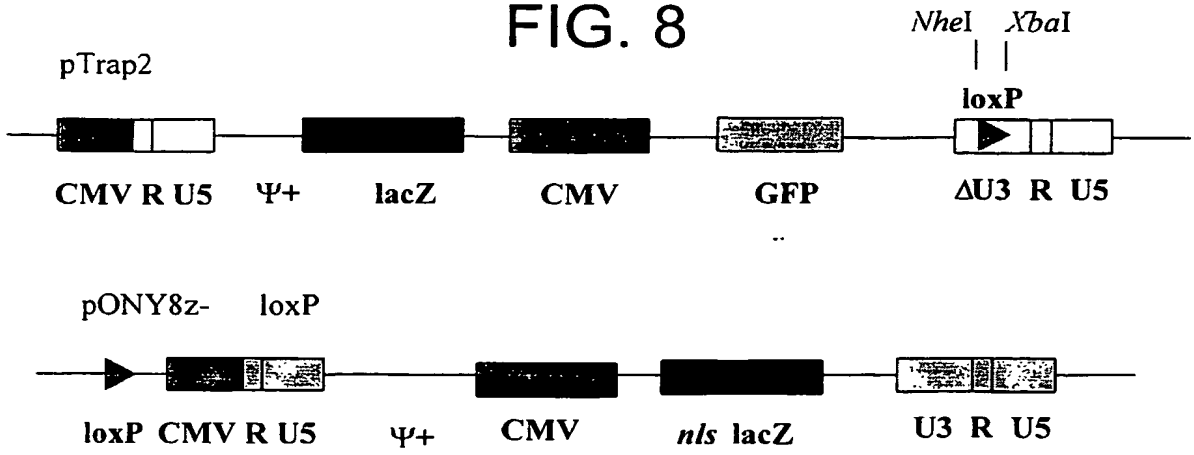
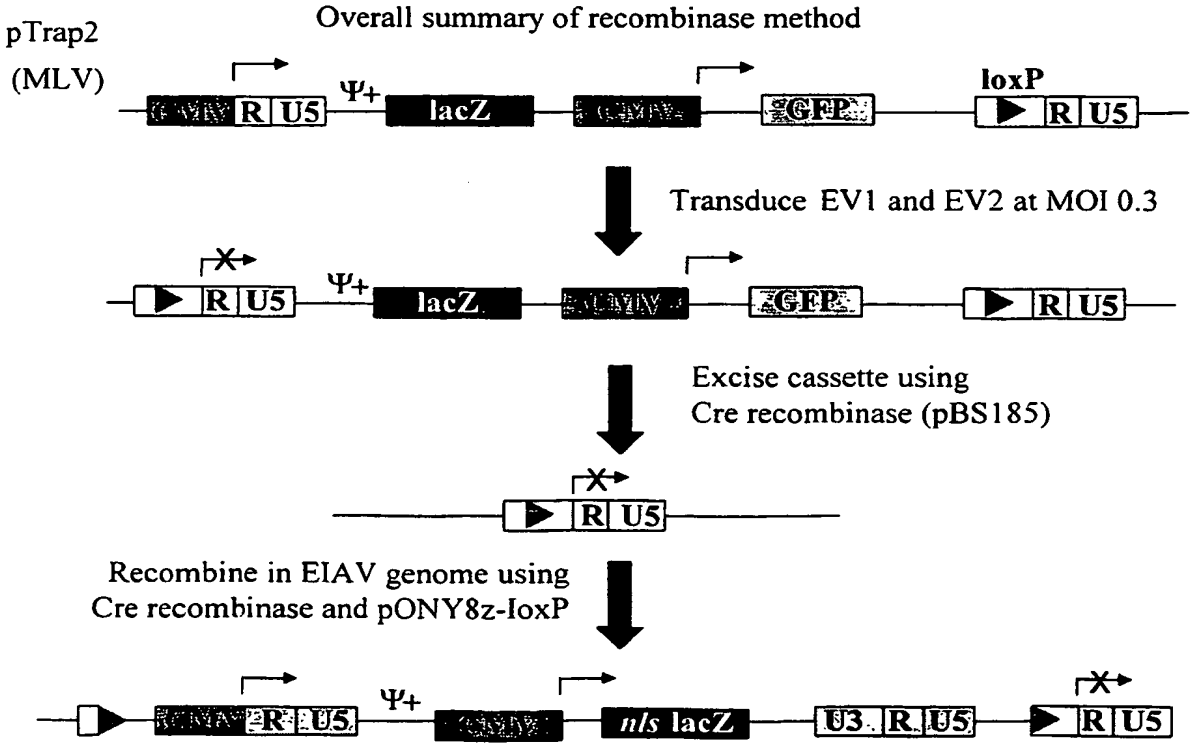

pTrap2
NheI XbaI
loxP
CMV R U5 Ψ+ lacZ CMV GFP ΔU3 R U5
pONY8z- loxP
loxP CMV R U5 Ψ+ CMV nls lacZ U3 R U5

FIG. 9


Overall summary of recombinase method
pTrap2
(MLV)
Ψ+
lacZ
GFP
loxP
R U5
Transduce EV1 and EV2 at MOI 0.3
Excise cassette using
Cre recombinase (pBS185)
Recombine in EIAV genome using
Cre recombinase and pONY8z-IoxP
nls lacZ
U3 R U5

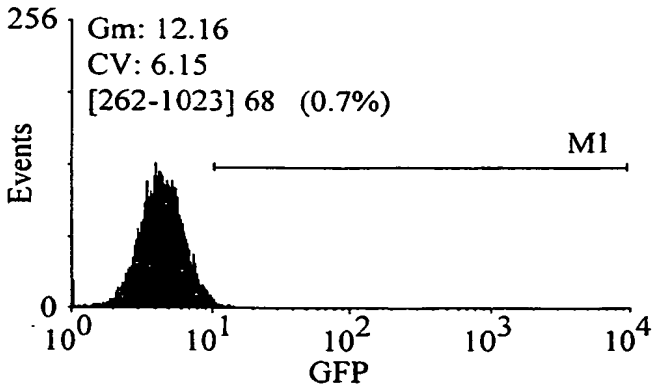


FIG. 10a

FACS analysis of EV1 packaging cells prior to transduction with Trap2 vector

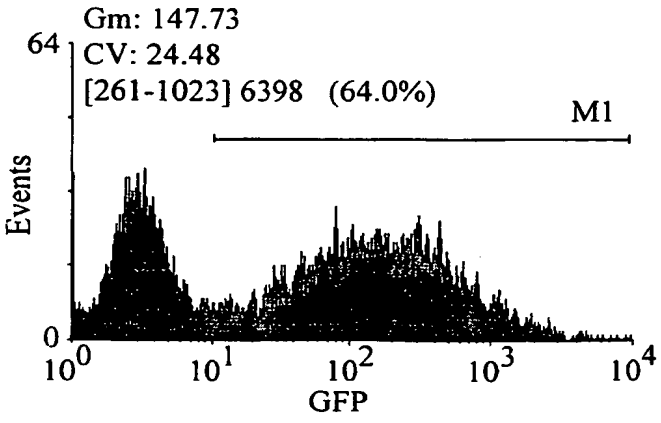


FIG. 10b

FACS analysis of EV1 packaging cell line transduced with Trap2 at an MOI of 0.3. A 5% top-slice of the highest expressers was carried out

FIG. 11

Validation of the ΔΔCt method for quantitation of GFP mRNA, relative to β-actin. A titration of total RNA from EV1 clone A was used. The difference in Ct values between the two assays is shown on the y-axis. The magnitude of the gradient must be <0.1 for the method to be valid. The gradient is 0.077, so the method is suitable.

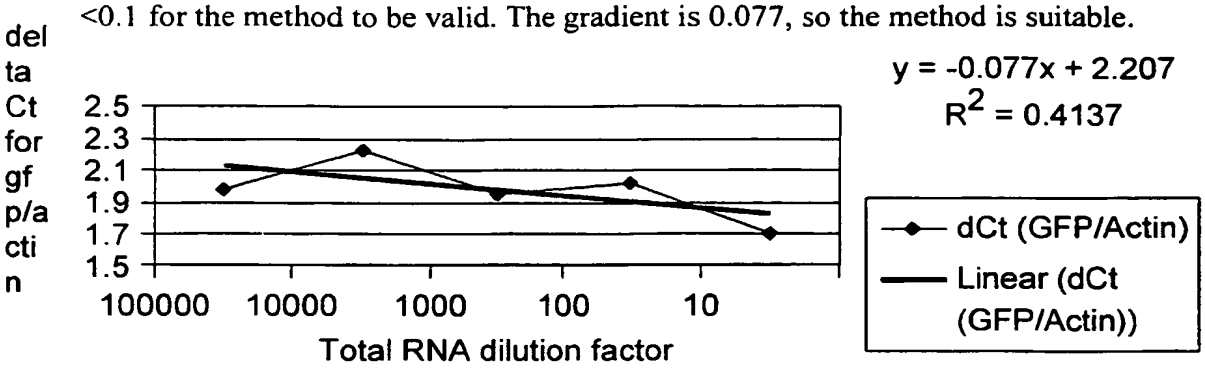


FIG. 12

Quantitation of GFP mRNA relative to control β-actin mRNA. EV2 TD cells are transduced with Trap2 at an MOI of 0.3 and are the calibrator sample with the ratio designated 1.0.

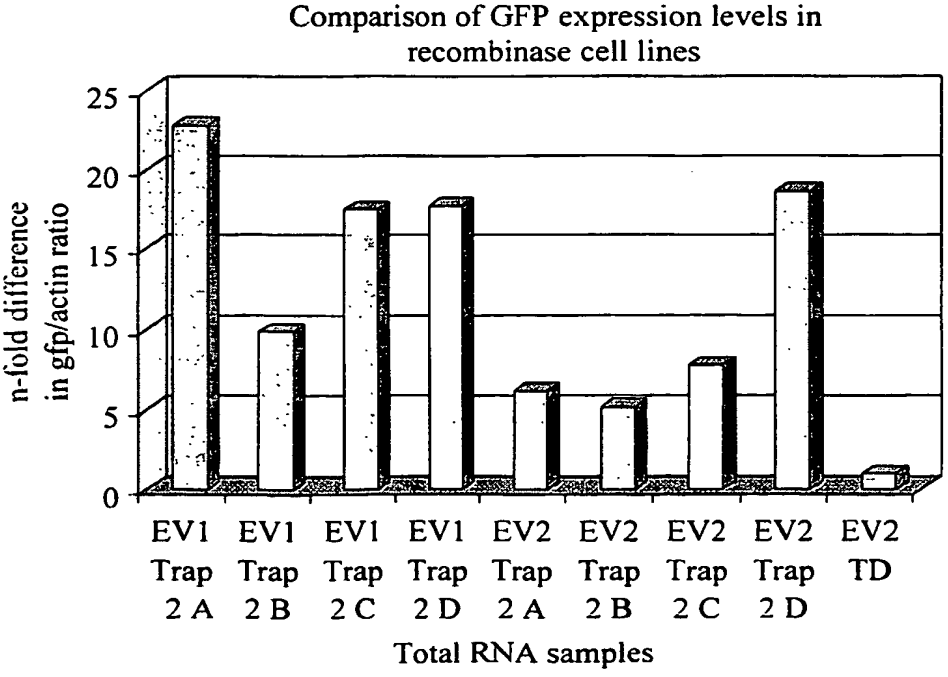
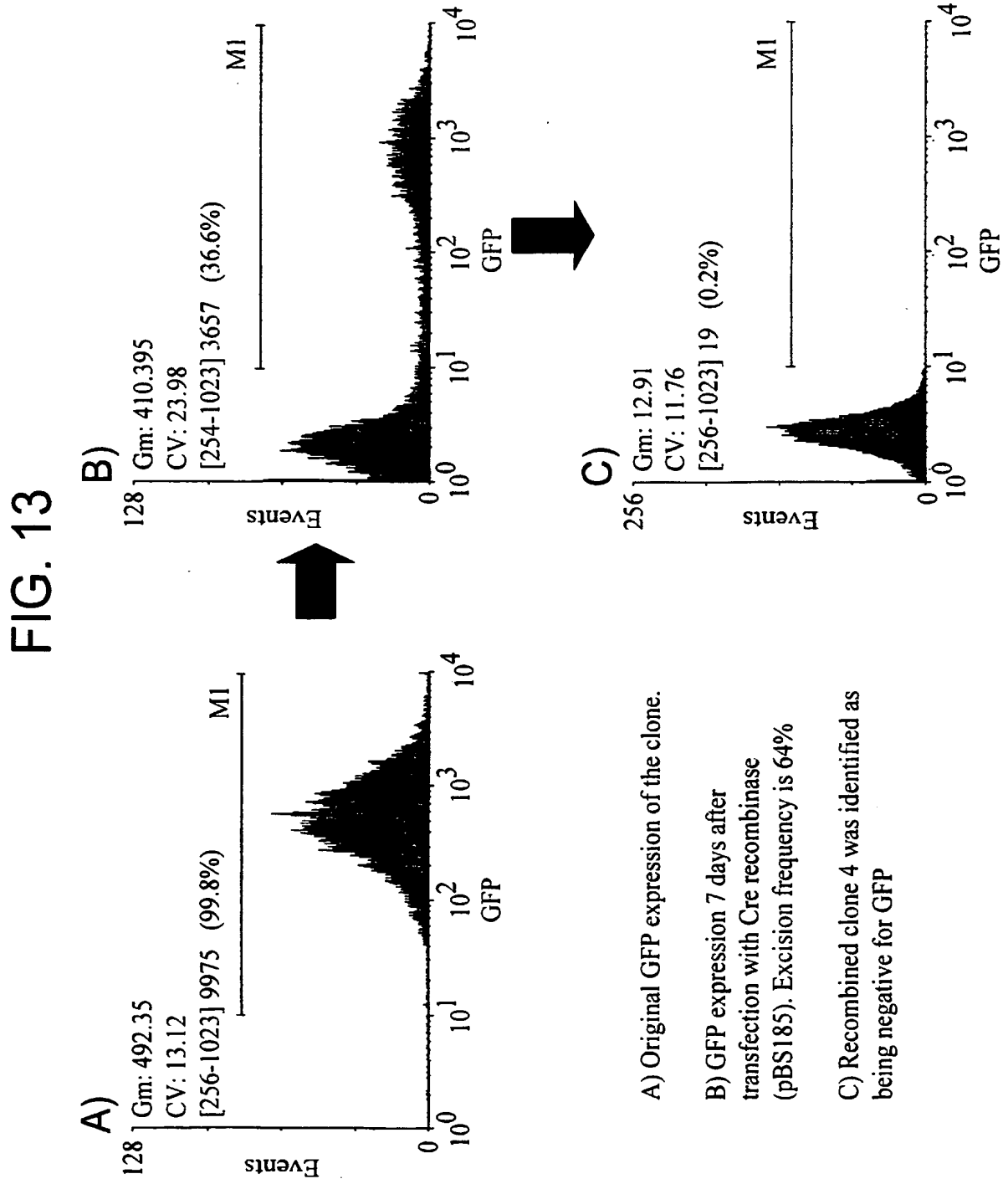


FIG. 13



A) Original GFP expression of the clone.

B) GFP expression 7 days after transfection with Cre recombinase (pBS185). Excision frequency is 64%

C) Recombined clone 4 was identified as being negative for GFP

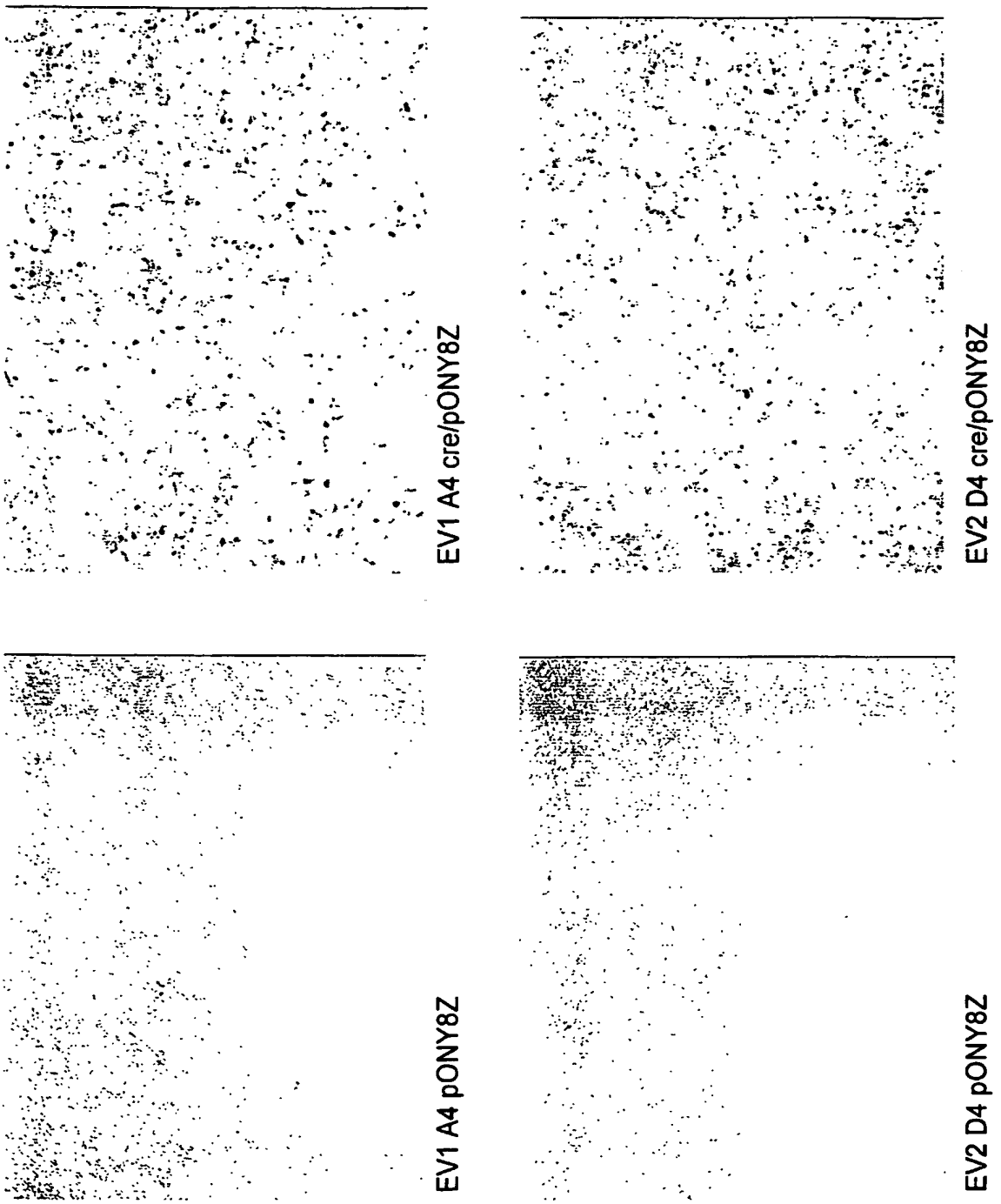

EV1 A4 cre/pONY8Z
EV2 D4 cre/pONY8Z
EV1 A4 pONY8Z
EV2 D4 pONY8Z

FIG. 14

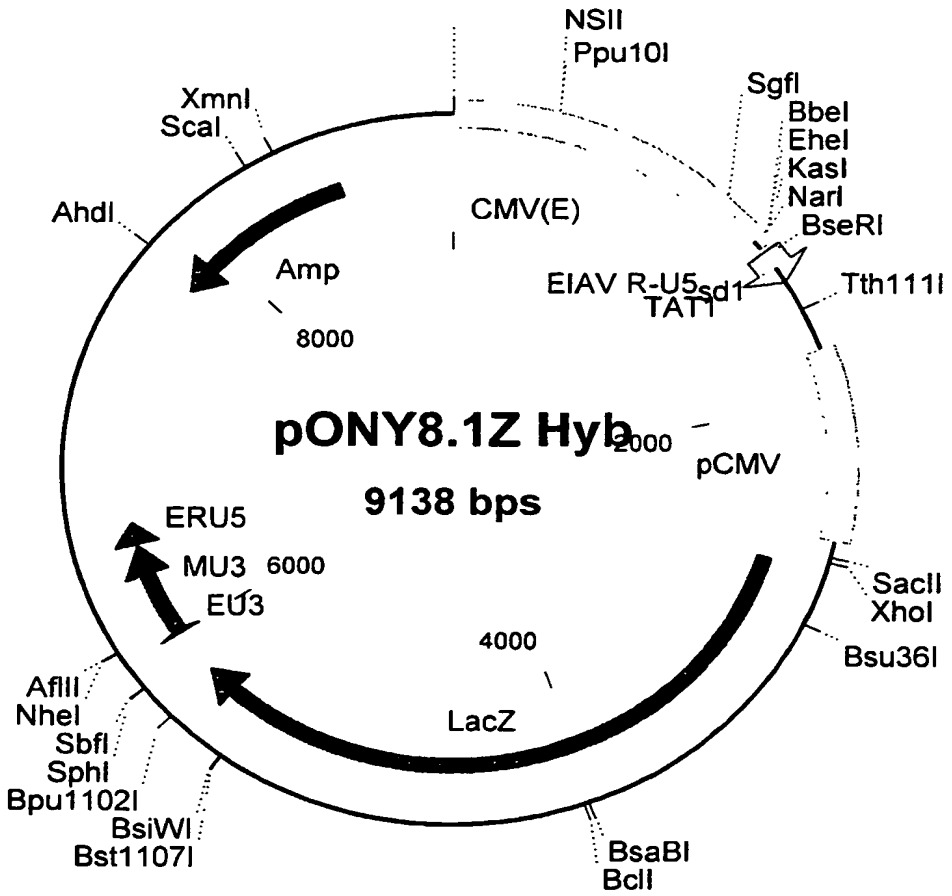

NSII
Ppu10I
Sgfl
BbeI
EheI
KasI
NarI
BseRI
Tth111I
SacII
XhoI
Bsu36I
BsaBI
BclI
Bst1107I
BsiWI
Bpu1102I
SphI
SbfI
NheI
AflII
ERU5
MU3
EU3
6000
4000
LacZ
pCMV
2000
pONY8.1Z Hyb
9138 bps
8000
Amp
Ahdl
XmnI
ScaI
CMV(E)
EIAV R-U5
TAT1
sd1

FIG. 15

FIG. 16

Alignment of leader and gag regions present in vectors pONY4Z, 8Z and ATG mutated 8Z vector. The later is referred to as pONY8ZA. The sequence aligned are from the NarI site in the leader to the XbaI site between the EIAV gag sequence and the CMV promoter. Sequences in the leader are shown in italic and a space is present upstream of the position of the gag ATG.

4Z	1 *cgcccgaacagggacctgagaggggcgcagaccctacctgttgaacctgg*
8Z	1 *cgcccgaacagggacctgagaggggcgcagaccctacctgttgaacctgg*
mutated 8Z	1 *cgcccgaacagggacctgagaggggcgcagaccctacctgttgaacctgg*
4Z	51 *ctgatcgtaggatccccgggacagcagaggagaacttacagaagtcttct*
8Z	51 *ctgatcgtaggatccccgggacagcagaggagaacttacagaagtcttct*
mutated 8Z	51 *ctgatcgtaggatccccgggacagcagaggagaacttacagaagtcttct*
4Z	101 *ggaggtgttcctggccagaacacaggaggacaggtaag*.at-gggagaccc
8Z	101 *ggaggtgttcctggccagaacacaggaggacaggtaag*.attgggagaccc
mutated 8Z	101 *ggaggtgttcctggccagaacacaggaggacaggtaag*.attgggagaccc
4Z	150 tttgacat-ggagcaaggcgctcaagaagttagagaaggtgacggtacaa
8Z	151 tttgacattggagcaaggcgctcaagaagttagagaaggtgacggtacaa
mutated 8Z	151 tttgacattggagcaaggcgctcaagaagttagagaaggtgacggtacaa
4Z	199 gggtctcagaaattaactactggtaactgtaattgggcgctaagtctagt
8Z	201 gggtctcagaaattaactactggtaactgtaattgggcgctaagtctagt
mutated 8Z	201 gggtctcagaaattaactactggtaactgtaattgggcgctaagtctagt
4Z	249 agacttatttcat-gataccaactttgtaaaagaaaaggactggcagctg
8Z	251 agacttatttcat-gataccaactttgtaaaagaaaaggactggcagctg
mutated 8Z	251 agacttatttcattgataccaactttgtaaaagaaaaggactggcagctg

4Z	298 agggat-gtcattccattgctggaagat-gtaactcagacgctgtcagga
8Z	300 agggat-gtcattccattgctggaagat-gtaactcagacgctgtcagga
mutated 8Z	301 agggattgtcattccattgctggaagattgtaactcagacgctgtcagga
4Z	346 caagaaagagaggcctttgaaagaacat-ggtgggcaatttctgctgtaa
8Z	348 caagaaagagaggcctttgaaagaacat-ggtgggcaatttctgctgtaa
mutated 8Z	351 caagaaagagaggcctttgaaagaacattggtgggcaatttctgctgtaa
4Z	395 agat-gggcctccagattaataat-gtagtagat-ggaaaggcatcattc
8Z	397 agat-gggcctccagattaataat-gtagtagat-ggaaaggcatcattc
mutated 8Z	401 agattgggcctccagattaataattgtagtagattggaaaggcatcattc
4Z	442 cagctcctaagagcgaaatat-gaaaagaagactgctaataaaaagcagt
8Z	444 cagctcctaagagcgaaatat-gaaaagaagactgctaataaaaagcagt
mutated 8Z	451 cagctcctaagagcgaaatattgaaaagaagactgctaataaaaagcagt
4Z	491 ctgagccctctgaagaatatct
8Z	493 ctgagccctctgaagaatatct
mutated 8Z	501 ctgagccctctgaagaatatct

FIG. 16 CONT'D

FIG. 17

Schematic representation of the structure of pONY 8.3G +/- vector genome plasmids